Review

Tannins: Current knowledge of food sources, intake, bioavailability and biological effects

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Tannins are a unique group of phenolic metabolites with molecular weights between 500 and 30 000 Da, which are widely distributed in almost all plant foods and beverages. Proanthocyanidins and hydrolysable tannins are the two major groups of these bioactive compounds, but complex tannins containing structural elements of both groups and specific tannins in marine brown algae have also been described. Most literature data on food tannins refer only to oligomeric compounds that are extracted with aqueous-organic solvents, but a significant number of non-extractable tannins are usually not mentioned in the literature. The biological effects of tannins usually depend on their grade of polymerisation and solubility. Highly polymerised tannins exhibit low bioaccessibility in the small intestine and low fermentability by colonic microflora. This review summarises a new approach to analysis of extractable and non-extractable tannins, major food sources, and effects of storage and processing on tannin content and bioavailability. Biological properties such as antioxidant, antimicrobial and antiviral effects are also described. In addition, the role of tannins in diabetes mellitus has been discussed.

1 Introduction

The term "tannin" was first introduced in 1796 [1] and came from the use of these compounds in tanneries. Tannins containing plant extracts have been used to process animal skin into leather since ancient times. Tannins were first defined by Bate-Smith and Swain in 1962 as water soluble, polyphenolic compounds with molecular weights ranging from 500 to over 3000 [2]. Characteristic features are their chemical identity reaction for phenols, and their capacity for precipitating proteins [3]. However, some tannins are not water soluble compounds; some can have molecular weights ranging from 3000 to over 30 000 [4] and can also be found in association with cell wall polysaccharides [5–8]. Tannins can therefore be defined as a unique group of phenolic metabolites of relatively high molecular weight having the ability to complex strongly with carbohydrates and proteins [9].

Tannins can be divided either by their chemical structure or by their solubility and extractability (Table 1). Concerning the chemical structure, tannins can be divided into four major groups, depending on the structure of the monomer: proanthocyanidins or condensed tannins, hydrolysable tannins, phlorotannins found in marine brown algae [10] and complex tannins.

Proanthocyanidins are polyhydroxyflavan oligomers or polymers. The monomeric flavanols differ in their hydroxylation pattern in ring A and B and in the stereochemistry of C-3. The most common monomers are the diastereomers (+)-catechin/(−)-epicatechin, (−)-gallocatechin/(−)-epigallocatechin and (+)-afzelechin/(−)-epiafzelechin, the respective oligo- and polymers are called procyanidins, prodelphinidins and propelargonidins. The flavanol monomers are usually linked by carbon-carbon bonds in the 4 → 6 or the 4 → 8 position (B-type proanthocyanidins). In some plants compounds with an additional C2 → C7 ether-linkage also occur (A-type proanthocyanidins) (Fig. 1). The

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Table 1. Tannins classifications on the basis of, A: chemical structure and constitutive monomer and B: extraction technique

A. Chemical structure
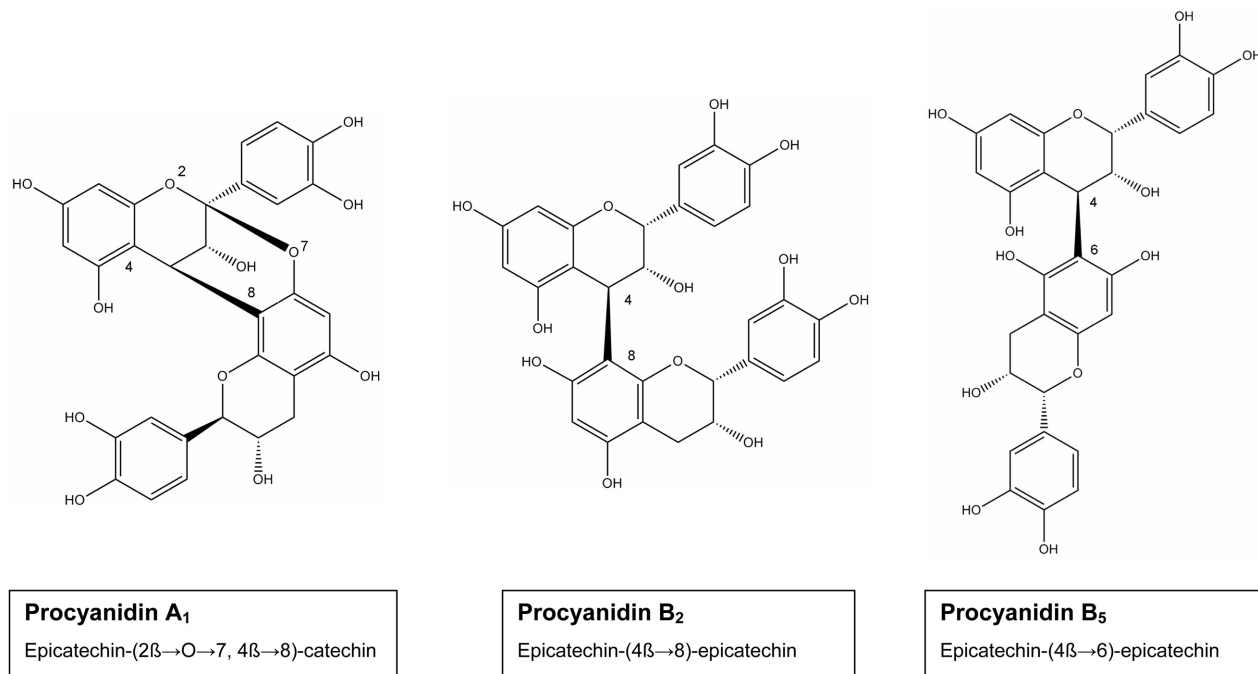
Group	Monomer	Monomer structure
Proanthocyanidins	(+)-catechin R1=OH, R2=H (+)-afzelechin R1=H, R2=H (−)-gallocatechin R1=OH, R2=OH	
	(−)-epicatechin R1=OH, R2=H (−)-epiafzelechin R1=H, R2=H (−)-epigallocatechin R1=OH, R2=OH	
Hydrolysable tannins	Gallic acid Hexahydroxydiphenic acid Ellagic acid (formed after hydrolysis)	Gallic acid Hexahydroxydiphenic acid
Phlorotannins	Phloroglucinol	
Complex tannins	Complex structures which contain structural elements of different tannin groups and other macromolecules	Phloroglucinol

B. Extraction technique

Group	Extraction technique	Analysis
Extractable tannins	Aqueous-organic solvents extraction	Spectrophotometric (Ferrum chloride, Folin-Ciocalteu, Vanillin-HCl) Chromatographic (HPLC, MALDI-TOF-MS, Thiolysis)
Non-extractable tannins	Acid and basic hydrolysis	Monomers quantification

degree of polymerisation varies over a broad range from dimers up to about 200 monomeric flavonol units [11]
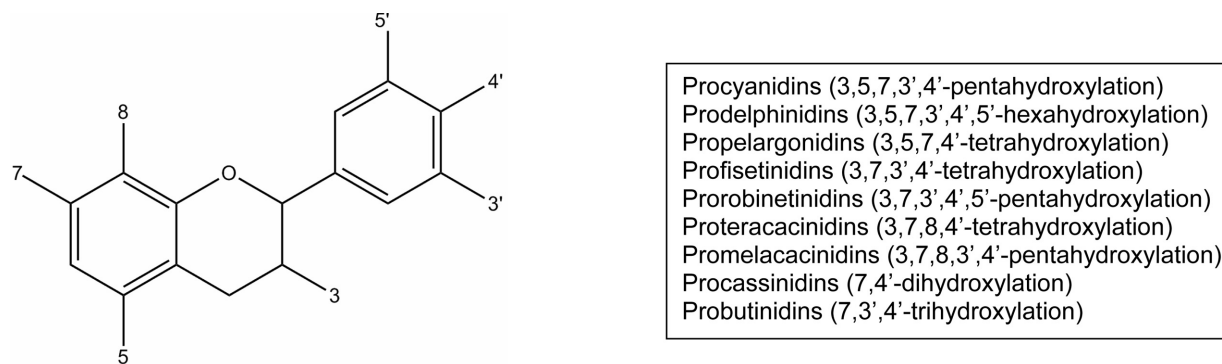
B-type proanthocyanidins can be classified according to the hydroxylation pattern(s) of the chain-extender unit(s) as: procyanidins (3,5,7,3′,4′-pentahydroxylation), prodelphinidins (3,5,7,3′,4′,5′-hexahydroxylation), propelargonidins (3,5,7,4′-tetrahydroxylation), profisetinidins (3,7,3′,4′-tetrahydroxylation), prorobinetinidins (3,7,3′,4′,5′-pentahydroxylation), proteracacinidins (3,7,8,4′-tetrahydroxylation), promelacacinidins (3,7,8,3′,4′-pentahydroxylation), procassinidins (7,4′-dihydroxylation) and probutinidins (7,3′,4′-trihydroxylation) (Fig. 2) [12].

In certain positions proanthocyanidins may sometimes be esterified with gallic acid or exceptionally with sugars.

Procyanidin A₁

Epicatechin-(2ß→O→7, 4ß→8)-catechin

Procyanidin B₂

Epicatechin-(4ß→8)-epicatechin

Procyanidin B₅

Epicatechin-(4ß→6)-epicatechin

Figure 1. Structures of simple dimeric procyanidins to demonstrate different linkage types in condensed tannins.

Procyanidins (3,5,7,3',4'-pentahydroxylation)
Prodelphinidins (3,5,7,3',4',5'-hexahydroxylation)
Propelargonidins (3,5,7,4'-tetrahydroxylation)
Profisetinidins (3,7,3',4'-tetrahydroxylation)
Prorobinetinidins (3,7,3',4',5'-pentahydroxylation)
Proteracacinidins (3,7,8,4'-tetrahydroxylation)
Promelacacinidins (3,7,8,3',4'-pentahydroxylation)
Procassinidins (7,4'-dihydroxylation)
Probutinidins (7,3',4'-trihydroxylation)

**Figure 2.** Proanthocyanidins classification in relation to the hydroxylation pattern.

Unlike hydrolysable tannins, proanthocyanidins react to insoluble phlobaphenes and red-coloured anthocyanidins when treated with acid, hence the name "proanthocyanidins" [13, 14].

Hydrolysable tannins are polyesters of a sugar moiety (or other non-aromatic polyhydroxy compounds) and organic acids. The designation "hydrolysable tannin" is due to the fact that these compounds undergo hydrolytic cleavage to the respective sugar and acid moiety upon treatment with diluted acids. In most cases the sugar component is glucose, but fructose, xylose, saccharose and seldom structures like hamamelose are also found. If the acid component is gallic acid, the compounds are called gallotannins. Esters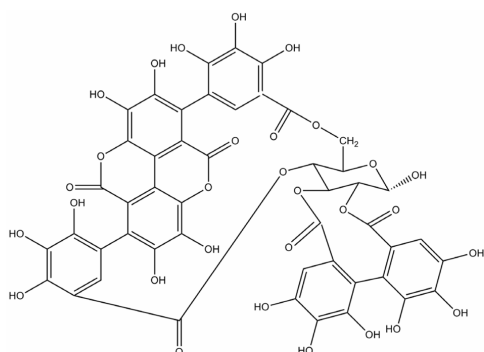 with hexahydroxydiphenic acid (forming ellagic acid when hydrolysed through elimination of water) are called ellagitannins (Fig. 3). Most ellagitannins are mixed esters both with hexahydroxydiphenic acid and gallic acid.

Furthermore, there are complex structures which contain structural elements of different groups. The so-called procyanidino-ellagitannins contains a (+)-catechin or (−)-epicatechin unit bound glycosidically to an ellagitannin unit. Upon hydrolysis, complex tannins yield (+)-catechin or (−)-epicatechin and gallic acid or ellagic acid [15]. Many further variations exist as tannins themselves can react with a large variety of molecules to form complex macromolecules.
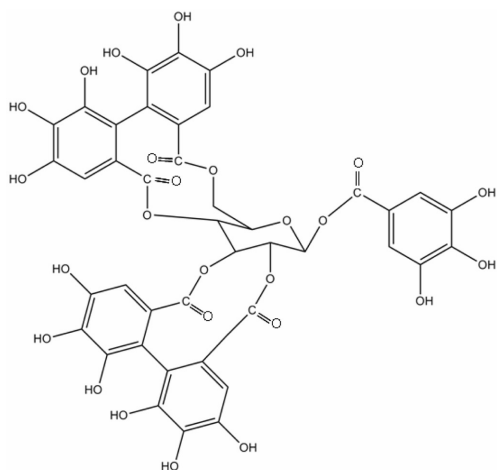
Tannins with special chemical structures are found in marine brown algae, the so-called phlorotannins (Fig. 4).

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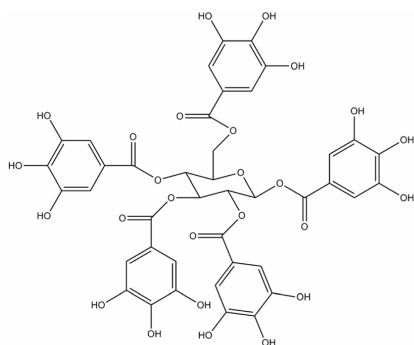
Gallotannin
Pentagalloylglucose
(1,2,3,4,6-penta-O-galloyl-ß-D-glucopyranose)

Ellagitannin
Punicalagin
(2,3-hexahydroxy-diphenoyl-4,6-gallagylglucose)

Ellagitannin
Casuarictin
(ß-D-glucopyranose esterified with gallic acid
and hexahydroxydiphenic acid)

Figure 3. Examples of structures of hydrolysable tannins.

These compounds are oligomeric or polymeric phloroglucinol (1,3,5-trihydroxybenzene) derivatives in which phloroglucinol units are connected by aryl-aryl bonds (fucols), ether bonds (phlorethols, hydroxyphlorethols, fuhalols) or both (fucophlorethols) (Fig. 4).

Independent from their chemical structure tannins may also be divided in soluble and insoluble tannins. Soluble tannins are oligomeric proanthocyanidins and relative low molecular weight hydrolysable tannins that can be readily extracted with different aqueous and organic solvents like aqueous methanol or acetone. High-molecular-weight tannins or tannins which form complexes with proteins or cell-wall polysaccharides are insoluble and remain in the residue of the extraction. Herbaceous forage legumes, for example, contain 55 to 86% of proanthocyanidins bound to protein [16]. However, other authors report lower amounts of bound proanthocyanidins (around 20%) in forages [17–19].

2 Extraction and analysis

2.1 Extraction

In general, tannins are extracted from plant material with ethanol, methanol, acetone or mixtures of these solvents with water. Lipophilic compounds may be removed with petroleum ether or dichloromethane. Low molecular weight compounds (proanthocyanidins with low degree of polymerisation or simple gallic acid esters) can be separated with ethyl acetate. Further separation is usually done with Sephadex LH-20 and RP materials with alcohol-water-mixtures or acetone-water-mixtures [12, 20]. All proanthocyanidins are sensible to light. For this reason light protection is recommended during extraction. Hydrolysable tannins are sensible to hydrolysis during extraction. Formation of artefacts may occur (*e. g.* formation of methyl esters during extraction with methanol) [13].

2.2 Analysis

Chemical investigation of tannins began rather late due to the lack of suitable methods for isolation and structure elucidation. Today there is no suitable method of analysing all the tannins contained in plants, especially highly-polymerised ones are difficult to analyse. Most of the studies found in the literature only deal with lower molecular weight tannins.

Tannins test positive for phenols (ferrum chloride, Folin-Ciocalteau). These reactions are not specific for tannins, as many other phenolic compounds also give positive results. Specific colour reactions which selectively detect esters of gallic acid or ellagic acid have been employed by Hartzfeld *et al.* [21]. Proanthocyanidins react to red reaction products when treated with mineral acids or with Vanillin-HCl [22]. The reaction of proanthocyanidins with dimethylaminobenzaldehyde leads selectively to blue reaction products

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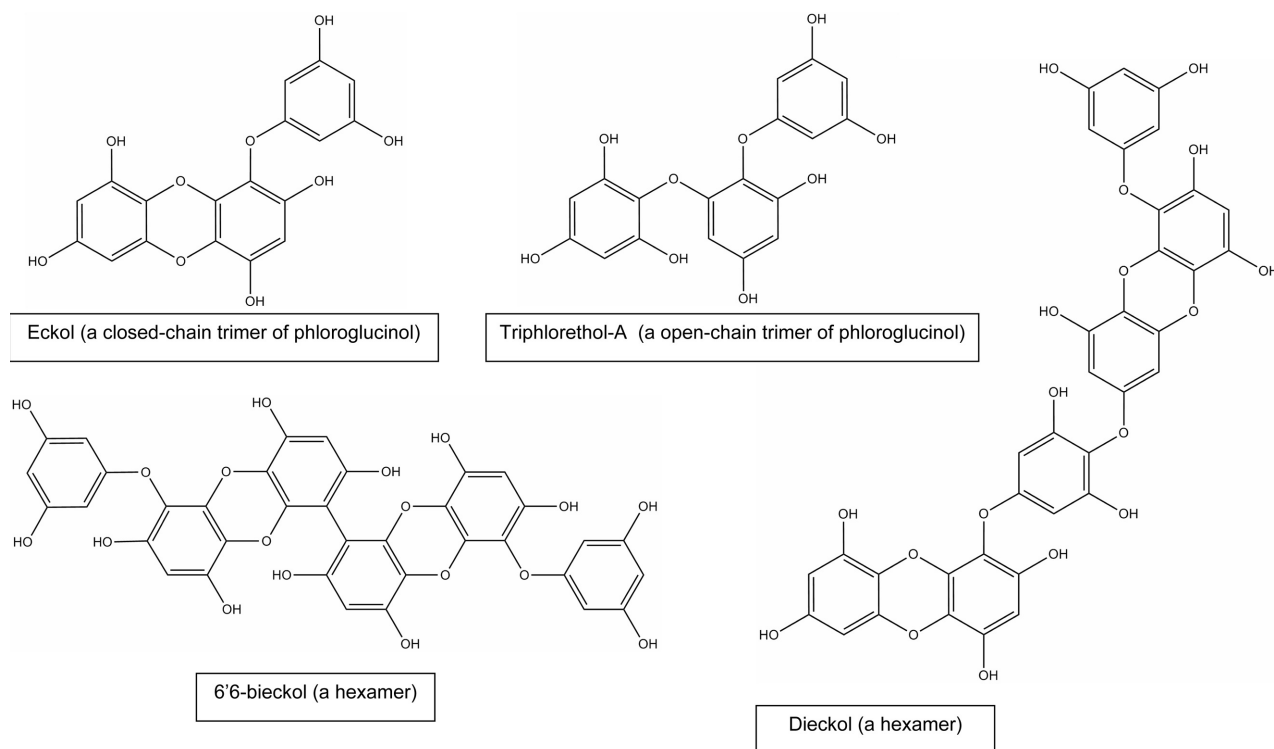
Figure 4. Examples of structures of phlorotannins.

[23]. These colour reactions are often used as group determinations for spectrophotometric quantification of tannins.

Numerous methods have been developed for qualitative analysis of low molecular weight tannins like proanthocyanidins up to the decameric level. These methods include paper chromatography, TLC, counter-current chromatography, centrifugal partition chromatography, several gel separation techniques and RP and normal phase HPLC [19, 24–26]. Normal phase HPLC has been used to evaluate proanthocyanidins upon a degree of polymerisation of six [26]. Most of these techniques are capable of adequately separating condensed proanthocyanidins up to decamers, but they are unable to resolve the more structurally diverse higher oligomers. Highly-polymerised proanthocyanidins are difficult to resolve by HPLC techniques since the number of possible isomers increases with degree of polymerisation [27].

Currently, there is no reference in the literature to a suitable method for analysis of highly-polymerised tannins. In the case of hydrolysable tannins, most reports only deal with easily extracted gallotannins and ellagitannins. Hydrolysable tannins are sometimes quantified in terms of free hydrolysation products like gallic acid or ellagic acid [28]. Strong acid hydrolysis has been employed to quantify high-molecular-weight tannins [24, 29]. Specific hydrolysable tannins can be determined after hydrolysis by several methods, *e. g.* galloyl esters [30], free and sterified gallic acid [31] and ellagic acid [32, 33]. Depolymerisation of high-molecular-weight condensed tannins in butanol yields red anthocyanidins that can be detected spectrophotometrically or chromatographed by RP-HPLC coupled with ESI-MS [27]. This method has been employed in several studies to estimate the proanthocyanidin content in foods [24, 29, 34, 35]. However, proanthocyanidin polymers are cleaved into dimers or trimers instead of monomers, thus presenting a risk of underestimation. Moreover, further reactions of the formed anthocyanidins with other hydrolysed compounds during the reaction may contribute also to the underestimate of the total content of proanthocyanidins

Thio-conjugation and extraction with methanol can be employed for analysis and determination of the degree of polymerisation of oligomers [35–37]. Various MS methods to determine the molecular composition of the monomeric units in proanthocyanidin oligomers are summarised by Lazarus *et al.* [38]. MALDI-TOF-MS is also used for the analysis of proanthocyanidins without prior derivatization. Indeed, even high-molecular-weight compounds can be separated and detected up to 100–200 flavanol units [11, 39]. However, the exact nature of the tannin molecules in food is still unclear in many cases.

3 Food sources, effect of storage and processing, and dietary intake

Several beneficial health effects have been attributed to intake of tannins, especially proanthocyanidins. To elucidate these effects requires sufficient information not only

on their activity, but also on their occurrence in the diet, so that adequate epidemiological correlations with the incidence of chronic disease can be established.

3.1 Food sources

Tannins are believed to be ubiquitous (Table 2, [40–96]), and it has been suggested that they account for a significant fraction of the polyphenols ingested in a Western diet. Only recently has a means been developed to measure proanthocyanidins with low polymerisation (<10 units) in specific foods [97, 98]. Also, a list of publications on foods containing detectable levels of proanthocyanidins can be found in the literature [46, 56]. Moreover, the USDA now has a database of proanthocyanidin contents of selected foods (http://www.nal.usda.gov/fnic/foodcomp/Data/PA/PA.html). Fruits including berries have been found to be major sources of proanthocyanidins in the diet, while in general terms vegetables are not an important source [34, 46]. Legumes, nuts and other minority cereals such as sorghum and barley contain proanthocyanidins, but they are not detectable in staple crops such as corn, rice and wheat. Wine, beer and some commonly consumed fruit juices are good sources of proanthocyanidins, whereas coffee is not. Proanthocyanidins tend to concentrate in the peel of fruits or the bran of grains. For example, the concentration of proanthocyanidins is higher in apples with peel than in apples without peel [46], being the degree of polymerisation higher in the peel than in the flesh [99].

As noted earlier, hydrolysable tannins can be found as gallotannins and ellagitannins. Gallotannins are not universally distributed in higher plants. They occur within clearly defined taxonomic limits in both woody and herbaceous dicotyledons. Ellagitannins are widely distributed in the lower Hamamelidae, Dilleniidae, and Rosidae [3] The occurrence of ellagitannins has been reported, among others, in almost all varieties of berries and their derivatives such as juices, jams and jellies: pecans, walnuts, brazil nuts, peanuts, cashews, blue plum, pomegranate (fruit and juice), red apples, navel oranges, pink and white grapefruit, tangerine, tangelo, peach, brown and green pear, white and red grapes, oak-aged wines, kiwi and beer (as an additive) (Table 2). Cereals could probably also be sources of hydrolysable phenols in the diet, since cereal bran contains significant quantities of phenolic compounds, benzoic and hydroxycinnamic acids, which are present in the plant cell wall mainly ester-linked to polymers. *Rubus* berries are known to be the best dietary sources of ellagitannins. Ellagitannin contents vary in different reports because extraction and hydrolysis conditions affect the ellagic acid yield [63, 100]. Seeram *et al.* [74] found that the total content of native ellagitannins and other sources of ellagic acid in pomegranate juice was 1770 mg/L, and the main ellagitannin was punicalagin. According to Kähkönen *et al.* [63], native ellagitannin content in acetone extracts of cloudberry, raspberry and strawberry were

1600–2400, 2500–2600 and 80–180 mg/kg respectively. The main ellagitannins in raspberry were sanguiin H-6 and lambertianin C [74]. Total ellagic acid content was 310 in pecans and 570 mg/kg in walnuts [61]. Pedunculagin was the major ellagitannin in walnuts [101].

3.2 Effect of storage and processing

There are only a very limited number of references to investigations into the influence of storage conditions on stability of tannins. The main reason may be that tannins were long regarded as food constituents without any nutritional value and without beneficial effects on health.

3.2.1 Effects of storage on the content of tannins

In raspberries which were stored at 4°C for 3 days and afterwards at 18°C for 24 h, mimicking conditions between harvesting and consumption, the levels of ellagitannins increased but the antioxidant capacity of the fruit was unaffected [102].

The effects of long-term freezer storage on berry ellagitannins were recently studied by Nohynek *et al.* [103]. The ellagitannins measured by HPLC were reduced by 25% in cloudberries and by 50% in red raspberries during 12 months storage. Häkkinen *et al.* [104] reported that ellagitannin content measured as ellagic acid after hydrolysis was reduced by 40% in strawberries and by 30% in red raspberries after nine months storage at −20°C, and according to De Ancos *et al.* [105] the amount of ellagic acid in frozen-stored raspberries decreased significantly (14–21%) in the course of one year.

Another study investigated the influence of sample drying and storage on the concentrations of hydrolysable tannins and ellagitannins in *Betula pubescens* leaves. Storage of fresh leaves at −20°C for 3 months produced no major changes in tannin content. Storage of freeze-dried leaves for one year at 4°C and at room temperature reduced the concentration of pedunculagin, one of the main ellagitannins in birch. Storage at room temperature also increased the levels of isostrictinin and 2,3-(S)-hexahydroxydiphenoyl-glucose, indicating possible degradation of tannins [106].

3.2.2 Effects on tannins during processing

There is rather more information available on the effects of different processing methods on stability of tannins. The aim of most studies has been to devise processing conditions which remove tannins from plants, since they are regarded as having an antinutritional effect, especially when the plants are used as animal feed. Most studies focus on proanthocyanidins.

Processing and cooking can also affect proanthocyanidin content in foods. Proanthocyanidin levels are high in fresh plums and grapes but are not detectable in prunes and raisins. This suggests that proanthocyanidins are degraded during the drying process [107] or polymerised to more

Table 2. Proanthocyanidin and hydrolysable tannin occurrence in different food sources.

Food group	Proanthocyanidins	Ellagitannins	Gallotannins	Reference
Cereals				
Barley	X			[40–42]
Sorghum	X			[43–45]
Legumes				
Beans	X			[47–51]
Chickpeas			X	[52]
Cowpeas	X		X	[53–55]
Lentils	X			[56]
Fruits				
Apple	X	X		[46, 56, 57]
Apricots	X			[46, 56]
Avocados	X			[46]
Bananas	X			[46, 58]
Blackberries	X	X		[46, 56, 59–61]
Blueberries	X	X		[46, 56, 59]
Cherries	X	X		[46, 56, 62]
Cloudberries		X		[63]
Cranberries	X	X		[46, 61, 63, 65]
Currants	X	X		[46, 56]
Dates	X			[46]
Grape	X	X		[46, 56, 60, 62, 66, 67]
Kiwi	X			[46, 56]
Lime		X		[68]
Mangos		X	X	[69, 70]
Medlar	X			[56]
Nectarines	X			[46]
Peaches	X			[46, 56]
Pears	X			[46, 56]
Persimmons	X		X	[71]
Pineapple		X		[59]
Plum	X			[46, 56]
Pomegranate	X	X		[56, 72]
Prune		X		[60]
Quince	X			[56]
Raspberries	X	X		[46, 56, 61, 73, 74]
Star fruit (*Averrhoa carambola* L)	X		X	[75]
Strawberries	X	X		[46, 56, 61, 59, 76, 77]
Vegetables				
Rhubarbs	X	X	X	[78, 79]
Squash	X			[46]
Beverages				
Cider	X			[80, 81]
Wine	X	X		[46, 82–84]
Beer	X			[46, 56, 85]
Tea	X			[56, 86, 87]
Cacao				
Cacao beans	X			[88, 89]
Chocolate liquor	X			[90]
Cocoa powder	X			[46]
Nuts				
Almonds	X			[46]
Cashew nuts	X	X		[46, 61]
Chestnut		X		[91]
Hazelnuts	X			[46]
Peanuts	X	X		[46, 61, 92, 93]
Pecans	X	X	X	[46, 61, 94]
Pistachio	X			[46]
Walnuts	X	X		[46, 95, 96]

highly-polymerised compounds, which may cause extraction and quantification to be incomplete.

The changes in the proanthocyanidin pattern of peaches after thermal processing/canning have been investigated by LC-MS. Thermal processing resulted in a 5–12% reduction of monomers up to pentamers. Approximately 30% of hexamers and heptamers were lost and octamers were no longer detected. Analysis of the syrup after processing revealed a migration of procyanidins into the syrup, which could account for the losses observed during the canning process. In canned peaches stored for 3 months, there was a time-related loss of higher oligomers, and compounds larger than tetramers were no longer observed [108].

Another study investigated the effect of different methods of production of grape juice on proanthocyanidin content. Cold pressing without maceration was the least and hot pressing after maceration at 60°C for 60 min the most effective method for extracting the flavan-3-ols. Pasteurisation had different effects: while the concentration of catechins increased in cold-pressed juices, it decreased in hot-pressed juices [109]. In grape seeds, proanthocyanidin content decreased by between 11 and 16% after heating at 100 and 140°C [110].

Several other studies have investigated the effects of processing methods on the tannin content of Sorghum grains. The effects of dehulling and storage of moist grains on tannin content were tested separately and in combination. The aim was to reduce the tannin content and to improve the nutritional quality of the grains. Abrasive dehulling of the grains, humidifying the grains with acetic acid, and storage for 7 days at 20°C proved to be the most effective procedure to reduce tannin content. Tannin was totally reduced and the *in vitro* digestibility of protein was increased to 87.5% [111].

Another study with Sorghum grains tried overnight soaking of sorghum in 2% $NaHCO_3$, soaking in different alkalis, ammoniation and autoclaving. Ammoniation was best for complete removal of tannic acid. Soaking the seeds in alkalis was also effective, as was soaking the sorghum seeds for 18 h in a mixed salt solution (containing 1.5% $NaHCO_3$/ 0.5% Na_2CO_3/0.75% citric acid) [112].

Processing of sorghum bran into cookies and bread has been found to significantly reduce procyanidin levels. This effect was more pronounced in the case of higher-molecular-weight polymers. Cookies retained more procyanidins (42–84%) than bread (13–69%). Extrusion of sorghum grains resulted in an increase in the levels of procyanidin oligomers (DP</ = 4) and a decrease in polymers (DP>/ = 6), possibly due to breakdown of the high-molecular-weight polymers into lower-weight compounds. It was concluded that processing changed the overall procyanidin content in sorghum, and also the relative ratios of the different molecular weights [113].

Another study examined how the tannin content of Faba beans (*Vicia faba*) was affected by domestic processes like soaking for 12 h, dehulling, ordinary cooking of whole as well as dehulled seeds under pressure for 15 and 25 min, and germination for 24, 36 and 48 h. The tannin content of the beans was reduced by 42–51% after soaking. Dehulled seeds showed a reduction of 70–73% in tannin content, and tannin content were also significantly reduced (76–81%) by cooking. Autoclaving for 25 min almost completely eliminated the tannins. Germination of seeds for 48 h produced a reduction of 90% [114].

Cowpea seeds (*Vigna unguiculata*) have been evaluated for their condensed tannin content and the effects of dehulling have been studied. Condensed tannin concentrations were 0.3–6.9 and 7.2–116 mg/g for whole cowpea seeds and seed coats respectively. Dehulling removed 98% of the tannin content of raw cowpeas [54].

Freezing, freeze-drying and every low temperature process seems to effectively prevent the condensed tannins in food/plant materials from degrading [110]. Mechanical processing of tannin-containing food should avoid dehulling of seeds as proanthocyanidins are mainly located in the seed coats. Different methods of thermal processing have been shown to cause significant loss of tannin content.

3.3 Dietary intake

A number of authors have estimated the daily intake of tannins based on food composition and consumption survey data. The mean intake for the general population (>2 years old) in the United States is estimated at 53.6 mg/day/person for a proanthocyanidin degree of polymerisation >2 [107], while in the Spanish population, proanthocyanidin intake is roughly estimated to range from several tens to several hundreds of milligrams *per* day [25]. Recently, Saura-Calixto *et al.* [34] determined and estimated the intake of highly polymerised proanthocyanidins in the Spanish diet at 450 mg/person/day. The estimated proanthocyanidin intake of the Spanish population based on the proanthocyanidin composition data published by Gu *et al.* [46] and the Spanish consumption survey data used by Saura-Calixto *et al.* [34] in the study mentioned below, is around 240 mg/person/day. This is lower than the value estimated by Saura-Calixto *et al.* [34], which means that some food items not included in the proanthocyanidin composition data from Gu *et al.* [46] may contribute to the proanthocyanidin intake of the Spanish population. Therefore, more extensive screening of proanthocyanidin content in food is needed.

There are limited data in the literature concerning ellagitannin and gallotannin intake. Some estimation can be made on the basis of the reported ellagic acid content of some foods. In Bavarian populations, Radtke *et al.* [115] estimated an intake of 5.2 mg/day of ellagic acid. Saura-Calixto *et al.* [34] estimated the intake of hydrolysable polyphenols (polyphenols associated with high-molecular-weight compounds, which includes hydrolysable tannins and other phenolic acids) in the Spanish population at

around 1250 mg/person/day. Nevertheless, these data may respectively underestimate and overestimate the hydrolysable tannin intake.

4 Bioavailability

Very little is known about the metabolic fate and bioavailability of tannins. To exert their biological properties, tannins have to be available to some extent in the target tissue. Therefore, the biological properties of tannins may depend on their absorption in the gut and their bioavailability. Absorption, bioavailability and metabolism of monomeric phenols have been extensively studied in both animals and humans [116–119], but little is known about the bioavailability of polymeric tannins and the results are controversial. It is unlikely that tannins with high molecular weight are absorbed intact. Almost ninety percent of the consumed procyanidins from apple juice were recovered in the ileostomy effluent and therefore would reach the colon under physiologic circumstances [120]. The degree of tannin polymerisation may have a major impact on their fate in the body. For example, more highly-polymerised proanthocyanidins typically present poor absorption through the gut barrier and limited metabolism by the intestinal microflora as compared to catechin [121]. Moreover, molecules appearing in blood or excreted in urine can be very different from those ingested.

4.1 Gastric and small intestine metabolism and absorption

There have been a number of *in vivo* and *in vitro* studies to evaluate the extent of hydrolysis of polymeric proanthocyanidins and the possibility of oligomeric molecule absorption in the small intestine. As regards gastric digestion, *in vitro* experiments suggest that procyanidins from chocolate are hydrolysed into bioavailable flavanol monomers in warm, acidic conditions which are thought to reflect those in the human stomach [122]. Nevertheless, Rios *et al.* [123] observed *in vivo* that there was no significant depolymerisation of cocoa procyanidins in the stomach and suggested that acid secretion in the stomach is buffered by the food bolus so that proanthocyanidins are exposed to much less acidic conditions. Tsang *et al.* [124] supports the view that oligomeric proanthocyanidins are not depolymerised into monomeric flavan-3-ols to any extent during passage through the stomach and gastrointestinal tract.

During small intestine digestion, high-molecular-weight proanthocyanidins can form complexes with protein, starch and digestive enzymes including pectinase, amylase, lipase, protease and β-galactosidase [125], resulting in the formation of less digestible complexes with digestive enzymes. Four types of linkages (*i. e.* hydrogen bonding, hydrophobic interactions, electrostatic and covalent bonding) are found

in proanthocyanidin-protein complexes, which are less soluble and less accessible to enzymes [126]. One mole of proanthocyanidins is reported to bind 12 moles of protein [125]; however, it has been suggested that small-molecule proanthocyanidins such as dimeric and trimeric proanthocyanidins present less complex formation activity and can be more readily absorbed [127]. On the subject of proanthocyanidin enzymatic digestion, Saura-Calixto *et al.* [34] observed *in vitro* that digestive enzymes were not able to release or increase the bioaccessibility of proanthocyanidins from the food matrix in food groups of the Spanish diet, suggesting that highly polymerised proanthocyanidins may reach the colon unchanged. *In vivo*, hydrolysis of procyanidins B2 and B5 to epicatechin has been reported in isolated rat small intestine [128]. The degree of polymerisation of proanthocyanidins has also been reported to decrease in rat small intestine [129]. Nevertheless, other *in vivo* findings suggest that oligomeric proanthocyanidins are not depolymerised into monomeric flavan-3-ols to any extent during passage through the stomach and gastrointestinal tract in rats, although trace quantities of procyanidin B1, B2, B3 and B4 dimers and the C2 trimer have been detected in urine [124].

Proanthocyanidin oligomer absorption in the small intestine has been studied by several authors. In Caco-2 cells, Deprez *et al.* [130] observed that (+)-catechin and a proanthocyanidin dimer and trimer had similar permeability coefficients, close to that of mannitol, a marker of paracellular transport. In contrast, permeability of a proanthocyanidin oligomer with an average polymerisation of six (MW = 1740) was approximately ten times lower. These results suggest that proanthocyanidin trimers and dimers could be absorbed *in vivo* and that polymer bioavailability is limited in the gut lumen [130]. Early studies from Jimenez-Ramsey *et al.* [131] demonstrated that proanthocyanidins soluble in water and ethanol are absorbed from the intestinal tract of chicks and are extensively distributed in all tissues and plasma, while proanthocyanidin fractions soluble in aqueous acetone but insoluble in water and ethanol are not bioavailable at all. Other studies have corroborated these findings. For example, Tanaka *et al.* [132] were able to measure the absorption of orally administered procyanidin B2 and procyanidin B3 in rat plasma. Baba *et al.* [133] likewise observed absorption and excretion in the urine of procyanidin B2 in rats, where a portion of the procyanidin B2 was degraded internally to (−)-epicatechin and to the conjugated and/or methlylated (−)-epicatechin metabolite. Indirect evidence suggests that at least some of the actions of proanthocyanidins are in part due to its absorption in the small intestine. Changes in the pattern of liver gene expression are observable as early as 5 h after oral administration of grape seed proanthocyanidins in rats [134]. The times of these responses suggest that they are triggered, at least in part, by proanthocyanidin molecules and not by their metabolites. Moreover, some *in vitro* effects

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of proanthocyanidin extracts are reproduced *in vivo*, whereas monomers have no activity or even display an opposite effect [135–137]. Other studies have reported no oligomeric proanthocyanidins in plasma and urine following proanthocyanidin ingestion [138], whereas monomers and aromatic acids derived from proanthocyanidin metabolism by gut microflora were detected in urine.

Regarding hydrolysable tannin bioavailability from the small intestine, few studies have evaluated the rate of hydrolysis into monomers (ellagic acid or gallic acid) during enzymatic digestion in the stomach and small intestine. Daniel *et al.* [139] reported that ellagitannins release ellagic acid upon hydrolysis in rats intestine. Nevertheless, the rate of absorption of hydrolysable tannin monomers (ellagic or gallic acid) has been extensively documented.

Gallic acid is permeated *via* a paracellular route in Caco-2 cells [140]. The intestinal absorption of gallic acid after oral administration in rats is relatively slow (t_{max}, 60 min) [141]. Similar results are reported in humans, with t_{max} of absorption around 1.27 h [142]. It was since suggested that there might be two different systems for gastric absorption of gallic acid: a rapid permeation system for intact gallic acid and a slow permeation system for conjugated derivatives [143]. The main metabolite of gallic acid absorption in humans is reported to be 4-*O*-methylgallic acid [144].

As regards ellagic acid absorption, no ellagic acid was recovered from blood or tissues of mice fed for 1 week on a diet containing 1% ellagic acid [145]. Nevertheless in humans some authors have detected ellagic acid in plasma between 0.5 and 3 h after oral administration of pomegranate juice (at a dose containing 24 mg of ellagic acid and 318 mg of ellagitannins), while no ellagitannins in intact forms were detected in plasma samples [146, 147]. The low concentrations of free ellagic acid in plasma have been attributed to its low solubility in water [148]. Moreover, ellagic acid has been reported to bind irreversibly to cellular DNA and proteins, which may also account for its limited transcellular absorption and its ability to form poorly soluble complexes with calcium and magnesium ions in the intestine [149]. Following absorption, ellagic acid undergoes conjugation, and conjugated forms with methyl, glucuronyl, and sulphate groups have been found in plasma and excreted in urine in humans [101, 150]. In this way, ellagic acid has been detected in plasma 1 h after consumption of 180 mL of pomegranate juice [147].

4.2 Large intestine metabolism and absorption

Another important site where tannins become available in the gastrointestinal tract is the large intestine. Most of the ingested tannins reach the colon, where they become fermentable substrate for bacterial microflora along with other non-digestible constituents [120, 121]. The abundant microbiota in the colon plays a critical role in the metabolism of tannins. After microbial enzyme metabolism of any tannin that reaches the colon, there are two possible routes available, breakdown of the original tannin structure into absorbable metabolites [151], or breakdown into non-absorbable metabolites (probably mid-molecular-weight tannins) which remain in the colonic lumen where they may counteract the effects of dietary pro-oxidants in the colon produced during colonic microbial metabolism.

Several authors have found that proanthocyanidins are highly metabolised by gut microbiota [34, 121, 152]. Polymeric proanthocyanidins were not analysable after 48 h of incubation with human colonic microbiota under anaerobic conditions [152]. Similar findings have been reported from proanthocyanidins associated in a food matrix [34, 153]. Phenylacetic, phenylpropionic and phenylbutyric acids are the main metabolites produced by the metabolisation of proanthocyanidins [152]. Moreover, in the aqueous phase of human faeces, aromatic acids have been found at much higher concentrations than monomeric flavonoids [154]. However, the total amount of the 14C label in these metabolites comprised 2.7% of the initial radioactivity from the substrate, suggesting only a small amount of microbial metabolism [152].

As many as 16 metabolites were detected in rat's urine after consumption of proanthocyanidin dimers, trimers and polymers isolated from willow tree catechins. These metabolites consisted of phenylvaleric, phenylpropionic, phenylacetic and benzoic acid derivatives, the total yields of which decreased significantly according to the degree of polymerisation of the precursor proanthocyanidins: catechin monomer (10.6%), dimer (6.5%), trimer (0.7%) and polymer (0.5%) [121]. In a study of human volunteers, 80 g of chocolate was consumed and from 584.8 mg of total proanthocyanidin intake (2.02 mmol) only 2.02 μmol was excreted in urine as microbial metabolites [155]. Thus it is possible for proanthocyanidins to pass through the entire gastrointestinal digestion largely intact, especially if their molecular weight is large. Nevertheless, some polyphenols may have the ability to bind strongly to various molecules in the colonocytes and dietary macromolecules in the colonic lumen, which could lead to underestimation if these are not recovered in the aqueous phase of human faeces.

There are no reports of intestinal bacteria that can degrade proanthocyanidins and their monomeric units, flavan-3-ols. Nevertheless, bacteria which are resistant to proanthocyanidins antibacterial properties have been isolated in recent years from the gastrointestinal tract ecosystem [156].

It has been reported that hydrolysable tannins are degraded to gallic acid, pyrogallol, phloroglucinol and finally to acetate and butyrate *via* sequential actions of different bacterial enzymes [149]. Gallotannins are easily degraded by bacteria, fungi and yeast, while the galloyl residues of galloyl esters in ellagitannins can be only hydrolysed in ellagitannins by microbes. Tannase (tannin acyl

hydrolase, EC 3.1.1.20), produced by a group of microorganisms such as fungi, yeast and bacteria, is active in galloyl residues of galloyls esters, as well as on hexahydroxydiphenoyl and other ellagitannins. It has both esterease and depsidase activities and particularly hydrolyses ester and depside bonds in gallotannins, releasing glucose and gallic acid. Unlike proanthocyanidins, colonic bacteria have been identified that are capable of metabolising hydrolysable tannins. Lactobacilli with tannase activity have been isolated from human faeces [158]. The presence of lactobacilli with distinct tannase activity suggests that gallic acid from gallotannins may be available during colonic fermentation. It is also clear from the results of *in vitro* experiments with intestinal content and *in vivo* animal studies that hexahydroxydiphenoyl groups in ellagitannins and galloyl groups in gallotannins can be hydrolysed [139]. Casuarictin and raspberry extracts incubated with caecal content increase the release of ellagic acid [139]. In rats fed with punicalagin, ellagic acid is transformed by rat microflora to 3,8-dihydroxy-6H-dibenzo (b,d)-pyran-6-one (urolithin B) derivatives, the total urinary excretion accounting for 0.7 – 52.7% of the ingested punilagin in rats [150, 159]. A pathway has been proposed for degradation of ellagitannin by human microbiota *via* hydrolysis to ellagic acid and its microbial transformation to urolithin B, which is detected from plasma as glucuronide after absorption. When a single dose of ellagitannin-containing foodstuff, *e.g.* strawberries, red raspberries, walnuts or oak-aged red wine, was taken by each group of human volunteers, the metabolite excretion ranged from 2.8 to 16.6% of the ingested ellagitannins, showing a large inter-individual variation between subjects within each group. High individual variation in the metabolite profiles is common for involvement of variable colonic microbiota [101].

Nowadays it is known for certain that tannins are partially metabolised and available for absorption at different sites in the gastrointestinal tract. For example, in the Spanish diet it has been estimated that about 40% of dietary tannins are bioaccessible in the small intestine, while 46% become bioaccessible in the large intestine, the principal sites of bioaccessibility being the small intestine for hydrolysable tannins and the large intestine for proanthocyanidins [34]. These data correlate with a higher bioaccessible antioxidant capacity observed *in vitro* [160]. Nevertheless, most of the molecules appearing in blood or excreted in urine are different from the ones ingested. Different biomarkers therefore need to be identified for tannin ingestion. Some biomarkers for consumption of polyphenols have been identified. For instance, isoferulic acid derivates have been proposed as biomarkers for the metabolism of caffeic acid derivates *in vivo* [161]. The metabolites of hydroxyphenylacetic acid derivates, hydroxyphenylpropionic acid derivates, vanillic, homovanillic, and hydroxyhippuric acid have also been proposed as biomarkers for consumption of different polyphenols including flavonoids and hydroxycinnamic acids [162,

163], and urolithin B as a biomarker for human exposure to dietary ellagitannins [101].

5 Biological effects

There are a lot of epidemiological data which suggest that tannin intake may prevent the onset of chronic disease [164]. Biological effects of tannins have been extensively studied using various *in vitro* or animal models; however, clinical data on humans is still limited or scarce. Note also that it is not clear as yet how or whether these complex polyphenolic compounds are absorbed from the gut, and that lack of precise knowledge of the fate of these compounds in the human body remains a major weakness in this area. Nevertheless it is believed that tannins may exert their biological effects in two different ways: (i) as an unabsorbable, complex structure with binding properties which may produce local effects in the gastrointestinal tract (antioxidant, radical scavenging, antimicrobial, antiviral, antimutagenic and antinutrient effects), or (ii) as absorbable tannins (probably low-molecular-weight) and absorbable metabolites from colonic fermentation of tannins that may produce systemic effects in various organs. Chemically, other authors suggest that tannins may exert their biological properties in three different ways: (i) by complexation with metal ions; (ii) through antioxidant and radical scavenging activities; or (iii) through their ability to complex with other molecules, including macromolecules such as protein and polysaccharides [165]. Very little is known about the biological effects of microbial metabolites of tannins. This question needs to be addressed in the near future to understand the significance of these compounds. Their residence time in human circulation is much longer than in the case of hepatic metabolites derived directly from plant phenolics [147]. Thus they may have an important role in chronic disease prevention.

5.1 Antioxidant and radical scavenging properties

Increasing attention is being given to the role of free radicals and other oxidants in the mechanism of action of many toxins, and in recent years also to their involvement in the pathophysiology of major chronic diseases. Oxidative stress reflects a disturbance of the prooxidant/oxidant balance of the body towards the former state and may arise from environmental or other external sources, or from the endogenous production of free radicals accompanying disease states [166]. Tannins are known to inhibit lipid peroxidation and lipoxygenases *in vitro*, and information has been accumulated over the past few years demonstrating their ability to scavenge radicals such as hydroxyl, superoxide, and peroxyl, which are known to be important in cellular prooxidant states [167]. Most of the activities of procyanidins, including the free radical-scavenging capacity, largely

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depend on their structure, particularly their degree of polymerisation, from which an increase in the antiradical power is observed with an increase in the degree of polymerisation up to seven [168].

Systemic effects related to mechanism of antioxidation have been under debate recently, since verification *in vivo* has not been successful due to inadequate biomarkers of effect [169–171]. However, local effects related to intact tannin structures are possible.

Procyanidins B1 and B3 have been evaluated as antioxidants for linoleic acid in aqueous systems [172], exhibiting stronger antioxidant activity than ascorbic acid or α-tocopherol. The higher the degree of polymerisation, the more radicals are scavenged *per* molecule [168, 173]. Uchida *et al*. [174] evaluated the radical scavenging action of galloylated condensed tannins by DPPH-radical and superoxide anions and by hydroxyl and peroxyl radicals, reporting a dose-dependent radical scavenging action.

However, it should be stated that, since the lower solubility of proanthocyanidins in the antioxidant capacity assay reaction medium, corrections for background absorbances are necessary. In that order, electron paramagnetic resonances spectroscopy has been used to evaluate the antioxidant capacity of polyphenols in cloudy apple juices instead of UV-visible measurements [175].

5.2 Antimicrobial and antiviral properties

The antimicrobial properties of tannins present in many plant foods have been well documented [176]. In general terms, tannins seem to affect bacterial growth in several mechanisms, such as inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation [177].

Complexation of metal ions in bacterial growth environment by tannins could also be a mechanism for exercising their antimicrobial properties [178]. In view of the importance of these metals to living systems, it is logical to assume that species which form strong complexes with them may well modify their biological activities. For example, many microorganisms produce siderophores (low-molecular-mass chelating agents that bind and solubilise iron). Siderophores employ three dihydroxybenzoyl rings to produce a charged octahedral triscatecholate Δ-cis complex. The mechanism of capture and binding of the transition metal is clearly very similar to that deployed by natural polyphenols in their complexation of such ions; therefore, there is presumably competition for substrate in the bacterial environment [179].

Bacteriostatic effects of berries due to anti-adhesion have been known for a long time. Cranberry juice has been associated with inhibition of *E. coli* adherence to uroepithelium [180–183]. Proanthocyanidins with unique molecular struc-

tures have been isolated from cranberry fruit, which exhibit potent bacterial anti-adhesion activity [184, 185]. Howell *et al*. [186] recently compared differences in structure and bacterial anti-adhesion activity of cranberry proanthocyanidins to proanthocyanidins from other foods, such as grape and apple juice. They found that only cranberry juice elicited bacterial anti-adhesion activity in human urine after consumption. Cranberry proanthocyanidins are also compositionally different from proanthocyanidins from other foods, characterised as they are by a series of oligomers based on repetition of the unit structure of catechin with one or more A-type linkages. The A-type linkages in cranberry proanthocyanidins are associated with anti-adhesion activity. Mice feeding experiments with cranberry proanthocyanidins suggest that a bioactive proanthocyanidin metabolite is present in urine, or properties of the urine are altered by the proanthocyanidins in such a way that adhesion is inhibited [187]. Recently Puupponen-Pimiä *et al*. [188] and Nohynek *et al*. [189] studied antimicrobial activity of eight Nordic berries and their phenolic extracts against several human gastrointestinal pathogens. They found that cloudberry and raspberry extracts, rich in ellagitannins, were the best inhibitors. Ellagitannins proved to be strong inhibitors of *Staphylococcus* bacteria, while *Candida albicans* and *Campylobacter jejuni* were sensitive only to cloudberry, raspberry and strawberry extracts rich in ellagitannins. In an earlier study Rauha *et al*. [190] also found that the widest bactericidal activity was expressed by berries belonging to the genus *Rubus* (cloudberry and raspberry).

Antiviral activity has also been observed on tannin extracts. The antiherpetic activities of hydrolysable tannins were dependent on the number of galloyl or hexahydroxydiphenoyl groups, while those of condensed tannins increased with the degree of condensation. The most cytotoxic tannins, however, were the most active ones [191]. Other authors have suggested that tannins may interfere with virus absorption. It has been demonstrated by radiolabelled virus particles of *Herpes simplex* virus that the antiviral effects of hydrolysable and galloylated condensed tannins were due to inhibition of virus absorption [192]. It has also been observed that several hydrolysable tannins significantly inhibit the cytopathic effects of human immunodeficiency virus, HIV, and the expression of HIV-antigen in human lymphotropic virus type I-positive MT-4 cells [193]. Anti-HIV activity was at least partly mediated by adsorption-inhibition, although complete inhibition of HIV-binding did not occur. Other authors have suggested that the inhibition in absorption may be derived from its binding to components of the viral envelope (in influenza A virus, parainfluenza virus, herpes virus type 1 and 2 and hepatitis A), resulting in inhibition of viral attachment and penetration of the plasma membrane [194]. It has also been demonstrated that ellagitannins and several condensed tannins are potent inhibitors of reverse transcriptase [195].

5.3 Antimutagenic activity

Non-mutagenic activity has been found for procyanidins with different degrees of polymerisation using the Salmonella mutagenesis assay system [196]. Antitumor activities have been shown in studies using purified ellagitannins. One such ellagitannin is sanguiin H6, which is found in raspberries and blackberries [197]. In other studies, raspberry extracts containing sanguiin H6 and lambertianin have presented antitumor activities. Unlike raspberry ellagitannins, ellagitannins from pomegranate present no antitumor activity.

Red wine polyphenols consisting of 28% proanthocyanidin units with a mean degree of polymerisation of 6.8 to 9.6% monomeric flavonoids or phenolic acids, influenced the colonic flora in F344 rats with azoxymethane-induced colon carcinomas. In polyphenol-treated animals the main bacterial strains were *Bacteroides, Lactobacillus* and *Bifidobacterium* spp., whereas the microorganisms predominantly identified in control-fed rats were *Bacteroides*, *Clostridium* and *Propionicbacterium* spp. Polyphenol-treated animals also had a consistently lower tumour yield compared to the control [198]. The total number of hyperproliferative crypts and of aberrant crypt foci was reduced by 50% in rats receiving 0.01% apple procyanidins in their drinking water. Showing that apple procyanidins alters intracellular signaling pathways, polyamine biosynthesis and triggers apoptosis in tumour cells [199]. Moreover, an apple polyphenols extract rich in proanthocyanidins has effectively suppressed the epidermal growth factor receptor phosphorylation, inhibiting the growth of human colon carcinoma cell line HT29 *in vitro* [200]. These compounds have also demonstrated to antagonise cancer promotion *in vivo* [201]. A tannin-rich diet may therefore have positive local health effects in the colon.

5.4 Antinutrient effects

The general protein-complexing effects of polyphenols can cause enzyme inhibition and therefore may interfere with gastrointestinal digestion and absorption of nutrients. There is a report of particular relevance to the present studies to the effect that crude or partly purified extracts of seed coats of *Phaseolus vulgaris* and *Vicia faba*, containing mainly polyphenols, inhibit sugar absorption in rat jejunum perfused *in situ* [202, 203]. Tannins were also found to reduce metallic ions such as $Cr(+6)$, $Fe(+3)$ and $Cu(+2)$ to $Cr(+3)$, $Fe(+2)$ and $Cu(+1)$ respectively when mixed with tannin solutions at room temperature [204], thus reducing their absorption.

5.5 Cardiovascular diseases

Cardioprotective effects of proanthocyanidins have been extensively reviewed by Rasmussen *et al.* [205]. Some epidemiological studies have associated intake of proanthocyanidins, among other dietary constituents, with reductions in cardiovascular disease risk factors (*e. g.* the French paradox). Subsequently, several animal studies have confirmed the *in vitro* indications of the cardioprotective potential of proanthocyanidins; they also showed significant decreases in plasma cholesterol levels and in the extent of atherosclerosis after feeding with proanthocyanidins. However the cardioprotective properties of purified proanthocyanidins still remain to be investigated.

5.6 Diabetes mellitus

Tannins may act on diabetes in two ways: (i) they may lower glucose levels by delaying intestinal glucose absorption and an insulin-like effect on insulin-sensitive tissues, and (ii) they may delay the onset of insulin-dependent diabetes mellitus by regulating the antioxidant environment of pancreatic β-cells.

Tannin extracts from various plants have been found to effectively inhibit intestinal enzymes. Inhibition of α-amylase and α-glucosidase activity has been reported by several authors [206, 207]. Tannins have been shown to effectively inhibit intestinal α-glucosidase activity with Ki values in the same range as with synthetic inhibitors (acarbose and voglibose), which are already being used therapeutically to control non-insulin-dependent diabetes mellitus [207]. Inhibition of α-amylase by phenolic extracts of pears, cocoa, lentils and tea has been reported [208]. Tannin-rich raspberry and strawberry extracts are effective inhibitors of both salivary and pancreatic amylase. The extent of inhibition of α-glucosidase is related to the anthocyanidin content (*e. g.* blueberry and blackcurrant), while the extracts that are most effective in inhibiting α-amylase (strawberry and raspberry) contain appreciable amounts of soluble tannins [209].

It has recently been suggested that tannins also act on cells by modifying or interacting with specific proteins of important intracellular signalling pathways and hence also affecting their role in improving hyperglycaemia. Grape seed procyanidin extracts have exhibited insulinomimetic properties *in vitro*, *e. g.* increasing the amount of GLUT-4 in membranes [210].

The role of oxidative stress in type I and type II diabetes mellitus is currently the object of intensive scientific research. Diabetes mellitus in experimental animals and humans is associated with reductions in antioxidants such as ascorbic acid, α-tocopherol and glutathione, suggesting that oxidative stress has a critical role in its pathogenesis. It is believed that insulin-dependent diabetes mellitus results from the destruction of insulin-producing pancreatic β-cells by multiple factors including viruses, chemical toxins and autoimmune response. Recent studies by Kaneto *et al.* [211] and Matsuoko *et al.* [212] have proven that reactive oxygen species lead to damage of β-cells through the induction of apoptosis and the suppression of insulin biosynthe-

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sis. Pancreatic β-cells are particularly susceptible to the deleterious effects of reactive oxygen species because of their low expression of the antioxidant enzymes genes as compared to other tissues.

Other authors have shown that grape seed procyanidin extract has similar anabolic properties to insulin; nevertheless it is less efficient at activating glycogen synthesis. Also, after chronic exposure it activates triglyceride turnover by simultaneously activating synthetic and degrading pathways and maintaining the total triglycerides content of adipocytes [213].

Furthermore, recent reports also seem to suggest that proanthocyanidins have a preventive effect against diabetes complications. Procyanidins derived from cacao have been found to inhibit diabetes-induced cataract formation in rats [214]. These inhibitory effects are probably due to the antioxidant capacity of proanthocyanidins

6 Concluding remarks

Tannins are widely distributed in nature and are present in almost all plant foods and some beverages; moreover, they are often the active compounds of the medicinal plants in which they occur. Nowadays, most of the interest in tannin intake derives from the possible implications for disease prevention. Reports of several *in vitro* assays demonstrate potentially significant interactions with biological systems evidencing antiviral, antibacterial, enzyme-inhibiting, antioxidant, radical-scavenging and antimutagenic properties.

Nevertheless, to determine the significance of tannins for human health, it is essential to know the amount of tannins consumed in the diet and their bioavailability. Literature data on the content and composition of dietary tannins are partial and insufficient to determine dietary intakes. In general terms, proanthocyanidins are principally found in fruits, especially berries, cocoa and some beverages like wine, beer and tea. Berries, legumes and leafy vegetables are the major sources of hydrolysable tannins.

The tannin content in food is principally affected by storage and thermal processes, where significant reductions are observed. However, it is not clear whether tannins are degraded during thermal processes or are polymerised to more highly-polymerised compounds, which could result in incomplete extraction or quantification.

The biological properties of any bioactive compound may depend on their bioavailability, nevertheless tannins bioavailability may differ quantitatively and qualitatively depending on the chemical analysis performed, since significant amounts of potentially bioactive tannins not extracted by traditional methods are sometimes ignored. Nowadays we know for certain that tannins are partially metabolised and available for absorption at different sites in the gastrointestinal tract, the colon being the principal site of absorption. It is therefore believed that tannins may exert their bio-

logical effects in two different ways: (i) as an unabsorbable, complex structure with binding properties which may produce local effects in the gastrointestinal tract (antioxidant, radical scavenging, antimicrobial, antiviral and the possible induction of intracellular signalling pathways and genes modulation) or (ii) as absorbable tannins (probably low-molecular-weight) and absorbable metabolites from colonic fermentation of tannins which may produce systemic effects in various organs.

Further research in this field is needed to focus on identifying and quantifying all the tannins contained in food, to further analyse the bioaccessibility and bioavailability of tannins, and to identify specific tannin molecules or metabolites that may have significant effects on human health. Data on tannin bioavailability may also be useful for the design and interpretation of epidemiological studies on the health effects of tannins.

The authors have declared no conflict of interest.

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